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Rearing Butterflies on Artificial Diets

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Abstract. Although the literature concerning artificial diets for moths is voluminous, few diets have been reported for butterflies. In this paper I have briefly examined the principles of diet formulation and give details of a diet which has proved successful for a range of species.

Introduction

Butterflies and moths are reared in captivity for a number of diverse reasons and it is therefore not surprising to find a range of techniques recommended in the literature. For the collector who requires only a few mint specimens for the cabinet, it may suffice to confine a wild-caught gravid female with potted foodplant and rear the resulting ova through to imagines thereon. Alternatively, the immature stages may be kept in closed containers and be regularly supplied with fresh foodplant, or simply "sleeved" - enclosed in a tube of netting - over a growing tree or shrub. Unfortunately, these methods are labour-intensive, require much space, and are therefore unsuitable for workers who require large numbers of insects. Further, there may be problems with foodplant availability and it is difficult to effectively control outbreaks of disease. Yet mass-rearing is a prerequisite for the study of most problems of applied entomology.

Bottger (1942) was the first to raise a lepidopteran, *Ostrinia nubilalis* (Huebn.), on artificial diet. The pink bollworm *Pectinophora gossypiella* Saunders, a well-known agricultural pest, was the first phytophagous insect reared on a wheat germ based artificial diet by Vanderzant and Reiser (1956a, 1956b). Since that time numerous diets have been developed for a wide range of moth species, especially those of economic importance; these diets have been collected into useful reference work by Singh (1977). However, few butterfly species have been reared on such diets - Singh (*loc. cit.*) lists only twelve species and these, too, are largely injurious to crops.

This paucity is probably due to a lack of commercial interest in butterflies, but experience has also shown that butterflies are in general somewhat more difficult to rear on artificial diets than are moths. But the difficulties are not insuperable and with a little experimentation most species can probably be reared in this manner. The purpose of this paper is not to present a universal pabulum for larvae, but rather to encourage experimentation.

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One of the first diets to show spectacular results with butterflies was that of David and Gardiner (1965). This was originally developed for the pest species *Pieris brassicae* (L.), but subsequently proved suitable for a range of other species with diverse feeding habits. Like many others, however, this diet is chemically quite well defined and therefore involves careful weighing and mixing of rather a large number of components. Although defined diets are essential for nutrition research, if the aim is simply to rear butterflies it is probably more convenient to use rather crude ingredients and accept that the results may not be reproduceable. This simplifies the diet and reduces costs.

The most successful of over forty diets I have tested since 1974 is detailed in Table 1. It is similar to that of Shaver and Raulston (1971) and has proved suitable for the species listed in Table 2. The diet produces reasonable results for such species, but is probably ideal for none and can undoubtedly be further improved and simplified. In the hope of encouraging this work, I shall try to explain the rationale behind the formulation, although the role of complex materials such as wheat germ and yeast extract is oversimplified in the following account.

Formulation of Diets

Like other animals, butterfly larvae require sources of protein, carbohydrates, lipids, vitamins, minerals and water. For the protein source many authors employ casein or milk powder, but I prefer soy flour since it is cheaper. Carbohydrates are supplied as sucrose or other sugars. Lipids are provided by wheat germ and the dried leaves, but corn oil may also be used at about 0.15% w/w. For some species the dried leaves may be omitted and in such cases corn or raw linseed oil must be added to prevent poor wing development. Vitamins may be added as a stock solution (as in the David-Gardiner diet), but it may be more convenient to use yeast or yeast extract. Minerals are best supplied by commercial salt mixtures such as Wesson salts. In addition, for most species one needs to add ascorbic acid (vitamin C) and choline.

Although the above may be nutritionally satisfactory, we have yet to induce the larvae to feed. Most species require a phagostimulant to bring about this behaviour. This is often some component peculiar to the foodplant, but may simply be sugar. The problem is usually solved by adding dried foodplant material at about 1.5 - 2.0% w/w, but much research is needed in this field. In some reports up to 40% plant material has been incorporated into the diet.

The diet must also possess the correct physical properties. It must contain water, but free water will usually result in the larvae drowning. It must be solid, but not too hard for the rather delicate mouthparts of young larvae. Clearly, some type of gelling agent is required. Gelatin is usually unsatisfactory due to its poor storage properties, and agar is therefore used

despite its higher cost. In fairly refined diets one should also add cellulose powder to provide bulk, texture and roughage.

We now have the basis of a diet, but there are a few useful extras. The agar produces a firmer, more brittle gel if potassium hydroxide is added. The pH of the diet is corrected by adding acetic acid. Since food for larvae is also food for microorganisms, potassium sorbate and methyl parahydroxybenzoate are added to reduce spoilage caused by fungal contamination. This reduces the need to sterilize the diet, but a formalin solution may be added providing care is taken to ensure that no trace remains in the diet when it is given to the larvae (it will evaporate from the warm diet). Finally, the risks of infection by the bacteria *Bacillus thurigiensis* may be reduced by adding the antibiotic aureomycin (chlortetracycline).

Preparation

How the diet is prepared may affect both its nutritional and physical properties. As a general rule, use fresh materials, finely ground, and mix thoroughly. The plant material should be carefully selected, washed and then oven-dried at 110 degrees C until it powders readily (10-30 min). The aim is to dry it as quickly as possible so that little volatile material is lost. The material is then ground (a coffee grinder is useful) and passed through a 0.5 mm mesh screen.

The agar is stirred into about 60% of the distilled water and then boiled for 3-10 min, with stirring. Ideally, this should be done in a steam bath to avoid burning the agar, but with care it may be done over direct heat. Allow the solution to cool to 75-85 degrees C.

Meanwhile, thoroughly mix the remaining solids in the rest of the distilled water (use a liquidizer if possible) and then add the cooled agar with further blending. The cooling serves to reduce decomposition of heat-labile substances, although the pH and protective action of other parts of the diet also help in this way. Next, add the liquids in order with 2 min blending between each addition. Finally, dispense the warm diet to containers, cover with clean paper towels, and leave to dry slightly for 24 h. The diet may then be stored almost indefinitely at 4 degrees C - bring to room temperature before use. If required, the diet may be autoclaved (a domestic pressure cooker is fine) without deleterious effects.

Containers and Rearing

The provision of suitable clean containers is an important part of rearing. For small larvae, plastic pots with card lids, such as creamers, are ideal and readily available from catering suppliers. Later one may use disposable drinking cups or glass jars (which may be sterilized for re-use). Do not overcrowd the larvae, and avoid excessive condensation by reducing numbers or fitting more permeable lids. Polystyrene cups are ideal for rearing, pupation and adult emergence due to the rough surface providing footholds.

It is advisable to start the rearing programme with ova, since larvae which have fed on normal foodplant may refuse the diet. Where possible, the ova should be removed from the plant using a fine brush, or by washing with water containing a little surfactant (0.2% Teepol) and bleach (1% sodium hypochlorite) at 20-25 degrees C. The latter also serves to surface-sterilize the ova, but may kill some. After 10 min wash twice with equal volumes of water, and collect the ova on filter paper. The ova are then transferred to the diet by means of a fine brush, or they may be stored in plastic containers until they hatch and the young larvae similarly transferred.

For many species the diet may simply be poured into the rearing containers to a depth of at least 0.5 cm, with a thin film on the sides. For others, especially edge-feeders, the diet may be shredded using a cheese grater. A further possibility, if the diet is not sterile, is simply to cut a piece from the block and press it firmly into the pot using clean paper towels. This provides a rough surface suitable for most feeding habits and also helps dry the surface slightly.

Newly-hatched larvae may wander for up to 24 h, and the acceptance varies from about 30-100% depending on species. Generally, members of the family Lycaenidae are the most difficult. In most cases progeny from insects reared on the diet settle more readily than their parents; one is evidently applying selection pressure.

The containers can be inverted to prevent frass fouling the surface of the diet although this may not be necessary. The temperature and light regime will obviously vary with the species and the aims of the investigator. A steady 20-25 degrees C in darkness is suitable for many species. The larvae should be transferred to fresh diet weekly, and one should aim to keep the density of larvae in each pot such that nearly all the diet is consumed in this period. For pupation, the pots may be turned lid uppermost so that the prepupae are formed there, or the container may be lined with paper or card (if polystyrene cups are not used).

Cannibalistic species present special problems, but many are well-suited to artificial diets since they settle readily; presumably a reflection of their catholic tastes regarding food sources. One may use a container for each individual larva, but this is costly and time-consuming. Shredded diet may be used to provide refuges in the food itself, so that a number of larvae may be kept in each container. This wastes diet, but it may be cheaper than providing more pots and filling each one with diet. Some larvae will be lost, and the optimum density of larvae per pot will have to be determined by experiments. Often high temperatures and humidity lead to increased cannibalism, so these variables should be kept in mind. This method is used for commercial production of both *P. gossypiella* (Mattoni, personal communication) and *Heliothis virescens* Fab. (Morton, unpublished) in the U.S.A.

An alternative is to pour the diet into trays and then press in a grid to provide a number of individual cells, much like an egg-box. Light diffusion grids and aluminium honeycombs are ideal for these purposes, and may be sterilized for re-use in strong bleach solution. This method has been used for *Heliothis* spp. (Raulston and Lingren, 1969; Raulston and Shaver, 1970), *P. gossypiella* (Morton, unpublished), and several butterflies.

Subsequent pairing and oviposition are unaffected by the use of artificial diet techniques, and these steps in the cycle may therefore prove to be the most difficult aspects of the whole rearing procedure. However, it is now possible to try to rear all year round, since the seasonal availability of larval foodplant is no longer a serious problem (although for most species some plants will still be needed to provide ovipositing stimuli). Similarly, one may tackle species which use plants that are not indigenous or readily available. Very large broods may be reared under almost identical conditions, facilitating investigation of problems in genetics and physiology. Finally, specimens may be easily obtained from a single wild female, thus reducing the possibility of damage to populations or habitat by excessive collecting. With regard to conservation of gene pools, it is now feasible to maintain laboratory populations considerably larger than those occurring in the wild (Morton, in print).

Those who feel unable or unwilling to prepare these diets themselves may be interested to know that plans are being laid by a private concern to market these products in aid of the Lepidoptera Research Foundation; the proceeds will be used to support field and laboratory research.

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Table 1

Composition of artificial diet for butterflies (listed in Table 2).

Solids	**% Composition (w/w)**
soy flour	7
wheat germ	6
yeast extract	6
sucrose	3.6
dried plant material	1.5
Wesson salts	1
ascorbic acid	0.4
potassium sorbate	0.2
methyl parahydroxybenzoate	0.15
aureomycin (veterinary grade)	0.023
agar	1.9
Liquids	
formaldehyde solution (10%)	0.43
potassium hydroxide (4M)	0.8
acetic acid (25%)	1.14
choline chloride solution (50%)	0.23
distilled water	to mass

Table 2

Species successfully reared on the artificial diet detailed in Table 1.

Hesperiidae

Pyrgus malvae L.
Erynnis tages L.
Thymelicus lineola Ochs.
T. sylvestris Poda (= *flavus* Breunnich)
Hesperia comma L.
Ochlodes venata Brem. & Grey

Papilionidae

Papilio machaon L.

Pieridae

Leptidea sinapis L.
Pieris brassicae L.
Artogeia rapae L.
A. napi L.
Anthocharis cardamines L.
Colias crocea Geoffroy
C. eurytheme Bsdv.
Gonepteryx rhamni L.

Lycaenidae

Thecla betulae L.
Quercusia quercus L.
Strymonidia w-album Knoch
Callophrys rubi L.
Lycaena phlaeas L.
Cupido minimus Fuessly
Celastrina argiolus L.
Plebejus argus L.
Aricia agestis Denis & Schiff.
Lysandra coridon Poda
L. bellargus Rott.
Polyommatus icarus Rott.

Riodinidae

Hamearis lucina L.

Heliconiidae

Agraulis vanillae L.

Nymphalidae

Limenitis camilla L.
Inachis io L.
Cynthia cardui L.
Vanessa atlanta L.
Aglais urticae L.
Polygonia c-album L.
Argynnis paphia L.
Mesoacidalia aglaja L.
Fabriciana adippe Schiff.
Clossiana euphrosyne L.
C. selene Denis & Schiff.
Eurodryas (Euphydryas) aurinia Rott.

Satyridae

Melanargia galathea L.
Hipparchia semele L.
Maniola jurtina L.
Aphantopus hyperantus L.
Pyronia tithonus L.
Coenonympha tullia Meuller
C. pamphilus L.
Pararge aegeria L.
Lasiommata megera L.